Resistance/epidemiology I

P568 Risk factors associated with trimethoprim-resistant bacteria in incident community urine samples

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Objectives: To evaluate the relationship between trimethoprim-resistant infection in community urine samples and possible risk factors said to be associated with urinary tract infection and drug resistance.

Methods: A case-control design used incident urine samples from July 1993 to December 1995. Cases were defined as subjects with trimethoprim-resistant Gram-negative bacterial (T_R) infection. One set of controls comprised subjects with trimethoprim-sensitive Gram-negative bacterial (T_s) infection. Another set of controls comprised those subjects who did not have T_R growth (i.e. (T_s+NG)). The risk factors include: age, sex, socio-economic status and prior exposure to antibiotics, hospitalization, corticosteroids, diabetes mellitus and oestrogen therapy. Exposures to risk factors in each group (T_R, T_s, NG) were determined using the MEMO record-linkage database. Cases were compared to each of the control groups (T_s and (T_s+NG)) by logistic regression.

Results: 13 765 incident urine samples were found (827 T_R, 2608 T_s and 10 330 NG samples). Generally, the regression analysis found increasing age, living in deprivation category 4 and prior exposure to trimethoprim to be significant predictor variables in both models. The final regression analysis for T_R versus T_s also found type of bacteria to be significant. Regression analysis for TR versus (T_s+NG) also found being female and exposure to oestrogen therapy to be significant.

Conclusions: This study demonstrates a strong link between community antibiotic prescribing and antibiotic resistance in bacteria. Prior hospital exposure is significantly associated with drug resistance, but disappears when adjusted in the multivariate model, suggesting confounding. It is shown by this study that bacterial antibiotic resistance is multifactorial and complex in nature.

P569 Hospitalization and community beta-lactam exposure are risk factors for antibiotic resistance in *Haemophilus influenzae* isolated from the respiratory tracts of adults

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Objectives: To determine risk factors for antibiotic resistance in respiratory isolates of *Haemophilus influenzae* (HI) in adults.

Methods: First respiratory isolates of HI detected in adults 15 years old in Tayside (April 1993 to December 1995) were identified. Hospitalization and community prescription of antibiotics in the 3 months prior to this were determined through computerized databases linked to the patients' unique identifier number. Cases were amoxycillin-resistant HI (HlamR) or co-amoxiclav-resistant HI (HlauR). Controls were HI sensitive (HIS).

Results: Of 412 respiratory isolates, 65 (15.8%) were HlamR, 8 (1.9%) were HlauR, 194 (47%) received antibiotics in the community and 169 (41%) had been hospitalized. Hospitalization was associated with HlamR or HlauR (OR 3.2, 1.8–5.6, $p<0.001$) even when patients with community-prescribed antibiotics were excluded (OR 4.5, 1.7–12.5, $p=0.0012$). Antibiotic exposure in the community was associated with resistance (OR 2.2, 1.2–3.7, $p=0.005$). Beta-lactam exposure (compared to prescription of any other antibiotic or no antibiotic) was associated with HIamR or HlauR (OR 2.3, 1.3–4.0, $p=0.002$) and this association persisted when only non-hospitalized patients were examined (OR 3.9, 1.6–9.8, $p=0.0016$).

Conclusions: Prescribing of beta-lactams in the community is associated with amoxycillin or co-amoxiclav resistance in HI. Hospitalization is also a risk factor for antibiotic resistance in HI and may reflect antibiotic or plasmid exposure in hospital.

P570 Current antimicrobial resistance of *Shigella* and *Salmonella* in Vilnius University Children's Hospital

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Objectives: To determine the rate of *Shigella* and *Salmonella* resistance to antimicrobial agents in 1997–98 and to compare the results with those from 1995.

Materials and methods: *Shigella* and *Salmonella* were isolated from stools of hospitalized children. The antibiotic resistance test was performed using disk diffusion.

Results: Resistance of the strains isolated in 1997–98 is given in the table.

Antibacterial agents	*S.sonnei*		*S.flexneni*		*S.enteritidis*	
	n	Resistance (%)	n	Resistance (%)	n	Resistance (%)
Ampicillin	106	54.7	107	86.0	246	2.0
Amoxicillin / clavulanic acid	69	1.5	62	21.0	103	1.9
Ceftazidime	10	0	22	4.6	100	1.0
Chloramphenicol	134	32.1	124	86.3	267	2.6
Gentamicin	135	0.7	124	0.8	265	1.5
Co-trimoxazole	134	81.3	124	46.0	267	6.4
Ciprofloxacin	60	0	54	0	92	0

Conclusions: The antibiotic resistance patterns of *Shigella* and *Salmonella* were different. The resistance of *Shigella* increased compared to the results from 1995. *Shigella* and *Salmonella* were sensitive to ciprofloxacin.

P571 Resistance investigations of *Enterobacter* spp. clinical isolates from patients of Slovak hospitals and university clinics in Innsbruck, Austria

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Objectives: We researched 32 multiresistant clinical isolates of genus *Enterobacter*, 13 isolates from Slovak hospitals and 19 from university clinics in Innsbruck, Austria. The purpose of this study was to compare mechanisms and transferability of aminoglycoside, beta-lactam and fluoroquinolone resistance in selected multiresistant isolates.

Methods: Antibiotic resistance was detected by the microdilution method (NCCLS, 1990). Identification of aminoglycoside-modifying enzymes (AGME) was done by the phosphocellulose paper-binding assay. The transferability of resistance was studied by bacterial conjugation with *E. coli* 3110 rif recipient. Resistance plasmids were isolated from donors and transconjugants according to Takahashi and Nagano.

Results: The most frequent material of isolation was urine. Resistance against aminoglycosides was more advanced in Slovak

isolates. A striking contrast was registered in resistance to isepamicin. The resistance rate to third-generation cephalosporins was the same in both Slovak and Austrian isolates. We have not observed resistance to cefepime, and fluoroquinolone resistance was detected only in isolates from Austria. In Slovak isolates the frequency of resistance transfer was relatively higher. Resistance to aminoglycosides apart from isepamicin was nearly always transferred; with beta-lactams there was lower frequency of transfer, and no transfer of resistance to fluoroquinolones was observed. In Slovak isolates we detected AAC (6′)-III, AAC (3)-11, APH (2″), and APH (3′)-VI AGMEs. In isolates from Innsbruck, production of AAC (6′)-I, AAC (3)-1, AAC (3)-II and APH (2″) was determined. We detected conjugative plasmids ranging from 75 to 90 Mda in both collections.

Conclusions: Austrian multiresistance isolates were characterized by relatively good susceptibility to isepamicin and cefepime. Slovak isolates were characterized by good susceptibility to cefepime and fluoroquinolones.

P572 Multidrug-resistant *Pseudomonas aeruginosa* sepsis in HIV⁺ subjects

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Objectives: To identify the risk factors for drug-resistant *Pseudomonas aeruginosa* (PA) sepsis in HIV subjects.

Results: In the study period, antibiotic resistance of 33 cases of PA sepsis changed as follows: CAZ from 12% (period A) to 43% (period B) (p=0.04; OR=6.37; period A versus B), CIP from 21% to 50% (p=NS), IPM from 21% to 45% (p=NS), PIP from 37% to 29% (p=NS) and GM from 42% to 52% (p=NS). At univariate analysis, cases statistically differed from controls with regard to: (1) presence of nosocomial infection (p<0.01); (2) previous broad-spectrum antibiotic therapy (p=0.01); (3) history of recurrent urinary infections (p=0.03); (4) number of CD4 lymphocytes <100/mm^3 (p=0.01); (5) high value of APACHE III score (p=0.01). Logistic regression analysis confirmed that previous antibiotic therapy and high APACHE III score independently predispose to the development of resistant PA sepsis.

Conclusions: These data emphasize the problem of resistant PA in HIV subjects, not only to new quinolones and IPM but also to CAZ.

P573 Surveillance of antimicrobial susceptibility of *Enterobacteriaceae* isolated in 10 hospitals in Portugal

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Objectives: To assess the prevalence of antimicrobial resistance in Enterobacteriaceae in Portuguese hospitals.

Methods: Ten hospitals located throughout the country tested all *Escherichia coli, Klebsiella* spp., *Enterobacter* spp. and *Proteus mirabilis* clinical isolates of 1997 against a selected group of antimicrobial agents.

Results: The number of tested strains and percentages of resistance against antimicrobials are summarized in the table (AM=ampicillin, CE=cephalotin, CZ=ceftazidime, CT=cefotaxime, TS=trimethoprim/sulfamethoxazole, CI=ciprofloxacin, GM=gentamicin).

Microorganism	AM	CE	CZ	CT	TS	CI	GM
E. coli (n= 8779)	48.9	12.4	0.9	0.3	32.1	16.2	7.0
Klebsiella spp. (n= 2197)	99.0	34.2	17.9	6.3	30.8	5.1	24.5
Enterobacter spp. (n= 1111)	96.1	89.1	26.1	24.4	16.2	11.0	14.0
P. mirabilis (n= 1602)	33.4	10.5	0.1	0.2	32.0	7.7	8.4

Conclusions: Despite the occurrence of some differences among hospitals, *P. mirabilis* and *E. coli* were the most sensitive species, while the most resistant were *Enterobacter* spp. In contrast to Gram-positive species, there is still a wide range of options for the etiologic therapy of infections caused by Enterobacteriaceae in hospitalized patients in Portugal.

P574 Susceptibilities of strains isolated from outpatient urine specimens in Italy

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The susceptibilities of 51 630 strains isolated from outpatient urine specimens in Italy were systematically estimated on the basis of data collected in 1996–97 from 37 laboratories and stemming from either the disk diffusion method or automated microdilution tests through data acquisition interfaces.

Escherichia coli (55.1% of isolates) showed 30.4% resistance to ampicillin (AMP) and 19.50% to piperacillin (PIP), while resistance to amoxycillin–clavulanate (AMX) occurred in only 2.9% of cases. These strains were resistant to cephalothin (CEP) in 11.9% of cases but to any third-generation cephalosporin (3GC) in less than 0.5%. The resistance rates to norfloxacin (NOR) and ciprofloxacin (CIP) were roughly 10%, and those to trimethoprim/sulfamethoxazole (SXT) and nitrofurantoin (FD) were 19% and 2.4%, respectively. As regards the aminoglycosides, the resistance rates to gentamicin (G), tobramycin (TOB), netilmicin (NET) and amikacin (AMI) were 4.5%, 2.8%, 1.1% and 0.4%, respectively.

Proteus mirabilis (7.7% of all isolates) showed 32.7% resistance to AMP, 17% to PIP, and 6.6% to AMX. The resistance to CEP was 16.9% and to 3GC not more than 2.4%. The resistance rates to NOR and CIP were around 6%. The resistance rate to SXT was 34.3%. The resistance rates to G, TOB, NET and AMI were 12.5%, 7.3%, 10.3% and 0.5% respectively.

K. pneumoniae (4.3% of all isolates) showed 89.2% resistance to AMP, 26.3% to PIP, and 3% to AMX. The resistance to CEP was 10.9% and to third-generation cephalosporins not more than 3.5%. The resistance rates to NOR and CIP were around 3%. The resistance rates to SXT and FD were 9% and 11.9%, respectively. The resistance rates to G, TOB and AMI were 2.6%, 2.3% and 0.8%, respectively.

Resistance to imipenem (IPM) proved extremely low among the Enterobacteriaceae mentioned, ranging from 0.2% (*E. coli*) to 1.3% (*P. mirabilis*).

E. fecalis (7.1% of all isolates) showed 1.1% resistance to ANT and AMX. Resistance to teicoplanin and vancomycin was roughly 0.5%. The resistance rates to NOR and CIP were 39.7% and 20.3%, respectively. The resistance rate to FD was 1.1%.

Worthy of note is the high incidence (3.7%) of *Pseudomonas aeruginosa*, with 9.2% resistance to ceftazidime, 2.9% to IMP, and 38.6% to CIP. However, only 2% of isolates proved resistant to all these substances, and 50% proved susceptible to all of them. The resistance rates to G, TOB and AMI were 33.4%, 30.6% and 7.9%, respectively.

P575 Nationwide prevalence study of *Pseudomonas aeruginosa* antimicrobial resistance in Spain

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Data regarding the antimicrobial susceptibility patterns of *P. aeruginosa* in a whole country without selection of patients and samples are scarce.

Objectives: To ascertain the present situation regarding antimicrobial resistance of *P. aeruginosa* in Spain.

Materials and methods: We carried out a point prevalence study in 136 hospitals by collecting all *P. aeruginosa* isolated during a previously selected week. All microorganisms were subjected to identification and susceptibility testing against 13 antimicrobial agents by the microdilution method (NCCLS). Serotyping was performed by standard procedures (IATS).

Results: A total of 1014 *P. aeruginosa* strains were studied. The origin of isolates was 68.5% nosocomial and 30.5% community. Wards of isolation were: ICUs 24%, surgery 26%, medical 38%, obstetrics, psychiatry and primary care 11%, and non-specified 1%. Origins of isolates were: respiratory tract 31%, wounds and abscesses 26%, urine 21%, blood 6%, ear 5%, and miscellaneous 11%. Percentages of resistance were: piperacillin/tazobactam 7%, meropenem 8%, amikacin 9%, piperacillin 10%, tobramicin 10%, ticarcillin 13%, imipenem 14%, ceftazidime 15%, cefepime 17%, ciprofloxacin 23%, aztreonam 23%, ofloxacin 29% and gentamicin 31%. The most frequent serotypes were 0:i (25.1%), 0:4 (21.6%), 0:11 (11.3%) and 0:2 (8.3%). The majority of resistant strains belonged to serotypes 0:12 and 0:11.

Conclusions: Resistance to quinolones and gentamicin is alarming and much higher than resistance to beta-lactams and carbapenems.Also remarkable is the high percentage of community-acquired isolates.

P576 Bactericidal activity of fourth-generation cephalosporins on multiresistant *Enterobacteriaceae* (ME). Interactions with amikacin (AM)

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Objectives: To compare the bactericidal activity of cefoselis (F), cefepime (C) and cefpirome (P) on ME alone or with AM.

Materials: A 5×10^6 CFU/mL log-phase inoculum of 4 *Escherichia coli*, 2 *Enterobacter clocae*, 2 *Enterobacter aerogenes* and 2 *Klebsiella pneumoniae* isolates—all resistant to third-generation cephalosporins, amikacin and quinolones—was exposed over time to 1×MIC and 4×MIC of F, C and P and to their interaction with 16 μg/mL of amikacin, i.e. equal to serum levels.

Results: The same ≥3 $\log_{10}$ decrease of the starting inoculum was observed with F, C and P with all *E. coli* isolates after 4 h of exposure and with all *K. pneumoniae* isolates at 24 h of growth, whereas it was not found with *Enterobacter* isolates. However, synergism was found with AM with all type of isolates after 4 h.

Conclusions: (1) Single fourth-generation cephalosporins act bactericidally on *E. coli*. (2) Synergism with AM is considerable.

P577 Antibiotic resistance in clinical isolates *of Haemophilus influenzae* from outpatients in Madrid, Spain

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Objectives: To study the antimicrobial susceptibility of 143 isolates of *Haemophilus influenzae* from outpatients residing in six different areas of Madrid, during 1997 and 1998.

Methods: We have studied the antimicrobial susceptibility of 143 strains of *Haemophilus influenzae* to 17 antimicrobial agents by agar dilution (Haemophilus Test Medium) and the production of ß-lactamase using the chromogenic cephalosporin method.

Results and conclusions: (1) 30.1% of the isolates were ß-lactamase producers (BLP). (2) For cefaclor the percentages of resistance and/or reduced susceptibility were 44.2% for the BLP and 24% for ß-lactamase-negative strains (BLN). (3) The MICs for the cephalosporins were higher in BLN strains, with MICs to ampicillin ≥2 mg/L, than in BLN strains, with MIC to ampicillin £1 mg/L. (4) The BLP strains with MIC for amoxycillin/clavulanate ≥4/2 mg/L were considerably more resistant to the cephalosporins than those with MIC for amoxycillin/clavulanate £2/1 mg/L. (5) The number of strains with reduced susceptibility to ampicillin, brought about by mechanisms other than the classic production of the enzyme, has increased. (6) All strains were susceptible to cefixime, cefpodoxime, ceftibuten, rifampin, nalidixic acid, and ciprofloxacin. (7) Azithromycin was more active than clarithromycin with 1.4% and 5.2% of non-susceptible strains respectively.

P577A How to detect ampicillin resistant *Haemophilus influenzae*

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Objects: To find a reliable routine method to detect ampicillin resistant Haemophilus influenzae strains.

Methods: The MIC's for 111 strains of *Haemophilus influenzae* were determined using the plate dilution method for penicillinV, penicillinG, ampicillin and ceftriaxone. The strains were categorized according to the ampicillin susceptibility, using the NCCLS breakpoints, susceptible: MIC≤1 mg/L, resistant. MIC≥4 mg/L. The strains were further tested using disk and tablet diffusion method, chocolateagar plates and ABbiodisks (penicillinV, penicillinG, arnpicillin, cefuroxime, and ceftriaxone; ABbiodisk, Solne, Sweden), and Neosensitabs (penicillinG, ampicillinlow, ampicillinhigh, cefuroxime, ceftriaxone; Rosco, Taastrup, Denmark). Production of ßlactamases was determined using BBL cefinase test (Becton-Dickinson, Cockeysville, USA).

Results: Ninetyone strains were fully susceptible (S) to ampicillin. Five strains ware intermediately resistant (1), of which 3 produced ßlactamases. Finally 15 strains were ampicillin resistant (R), 11 of these produced ßlactamases. Given as median (range) in mm and zone diameter of 0 indicating growth to the edge of the disk, zone diameters for penicillin V using ABbiodisks were S: 15 (027), 1: 0 (013) and R: 0 (012). Ampicillin ABbiodisks, Ampicillin low and high Neosensitabs and cefuroxim ABbiodisks and Neosensitabs showed low or no discrimination between ampicillin susceptible and resistant strains. All strains were susceptible to ceftriaxone.

Conclusion: The most sensitive disk/tablet diffusion method to detect ampicillin resistant strains was the use of penicillin V disks.

P578 Antibiotic resistance evolution of enteric *Salmonella* and susceptibility pattern

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Objectives: To study the evolution of enteric *Salmonella* antibiotic resistance during January 1994 to October 1998 in an outpatient population.

Materials and methods: 290 enteric *Salmonella* strains were isolated from coproculture during a 5-year period. Identification and antimicrobial susceptibility were determined on the microScan system (DADE) according to the NCCLS recommendations. Serotyping of strains isolated was tested using polyvalent grouping antiserum and group-specific antisera for flagellar and somatic antigens (Difco and Pasteur).

Results: The most frequent antibiotic resistance patterns were as follows: 140 strains (48.27%) showed susceptibility to all antibiotics tested; 26 strains (8.96%) showed resistance to piperacillin, ampicillin and ticarcillin; 16 strains (5.51%) showed resistance to piperacillin, ampicillin and ticarcillin, ticarcillin/K clavulanate, amoxycillin/K clavulanate and tetracycline; 20 strains (6.89%) showed resistance to piperacillin, ampicillin, ticarcillin, ticarcillin/K clavulanate and tetracycline; 12 strains (4.13%) showed resistance to piperacillin, ampicillin, ticarcillin, T/S and tetracycline; 9 strains (3.10%) showed resistance to piperacillin, ampicillin, ticarcillin, ticarcillin/K clavulanate, T/S, amoxycillin/K clavulanate and tetracycline; 8 strains (2.75%) showed resistance to tetracycline; 8 strains (2.75%) showed resistance to aztreonam; 5 strains (1.72%) showed resistance to T/S and tetracillin; 3 strains (1.03%) showed resistance to ampicillin, ticarcillin, T/S and tetracycline; 3 strains (1.03%) showed resistance to piperacillin, cefalotin, ampicillin and ticarcillin; 40 strains (13.79%) showed a different resistance pattern.

Conclusions: In practice, about 100% of strains were susceptible to amikacin, cefotaxime, ceftriaxone and ciprofloxacin. Less susceptibility was found to ampicillin, piperacillin, tetracycline, ticarcillin, ticarcillin/K clavulanate and tetracycline. Almost 50% of the strains studied were susceptible to all the antibiotics tested.

P579 Antibiotic pressure and resistance pattern of prevailing bacteria in Tartu University Clinics

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Objectives: To determine the susceptibility to the most frequently used antibiotics of bacteria most commonly found from clinical specimens in University Clinics.

Methods: Bacteria (n=1251) isolated from patients of University Clinics during January to July 1998 were included. Antibiotic usage in Tartu University Clinics and in all Estonian hospitals in 1995 and 1997 was calculated as defined daily doses per 100 bed-days (DDD/100).

Results: The top three antibiotics used in University Clinics in 1995 were doxycycline, ampicillin, and gentamicin (26%, 24% and 13% of total 47.6 DDD/100). This top three was the same in different types of departments, except ICUs, where cephalosporins were used more than doxycycline. In 1997, the use of gentamicin was decreased due to partial replacement with newer aminoglycosides. In other Estonian hospitals the use of antibiotics was similar. The most frequently isolated bacteria in Tartu Clinics were: S. *aureus* (17%), *E. coli* (15%), *Pseudomonas* spp. (11%), *Klebsiella–Proteus–Enterobacter* (KPE, 10%), coagulase-negative staphylococci (CNS, 9%), ß-hemolytic streptococci (8%), *Acinetobacter* spp. (7%), and enterococci (3%). In internal and surgical departments, *E. coli* and S. *aureus* dominated but in ICUs *Pseudomonas* spp., *S. aureus* and *Acinetobacter* spp. prevailed. Percentage resistance to ampicillin, doxycycline and gentamicin was as follows: *S. aureus* 99, 57, ND; *E. coli* 51, 57, 5; *Pseudomonas* spp. ND, 100, 22; CNS 97, 48, ND; *Acinetobacter* spp. ND, 85, 83; KBE 78, 53, 45; enterococci 13, 64, ND.

Conclusions: Resistance to ampicillin, doxycycline and gentamicin of the prevailing bacteria in our hospitals was very high. One possible reason is selective pressure owing to frequent use of these antibiotics during recent years.

P580 French survey of antibiotic susceptibility of pathogens responsible for infections in intensive care units

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Prudent use of antibiotics requires good knowledge of antibiotic susceptibility levels for the most frequent pathogens. We have conducted a multicenter study of this antibiotic susceptibility level for the clinical strains isolated from patients hospitalized in 18 hospitals. All patients were hospitalized in intensive care units or hematology wards. The survey took place from 1 January 1998 to 30 June 1998.

1113 clinical isolates were studied from: urines (337), blood cultures (344), pulmonary and bronchial samples (344), and deep pus (48). Enterobacteriaceae were the most often isolated (*E. coli* (248), *E. aerogenes* (39), *P. mirabilis* (33), *K. pneumoniae* (28), *E. aerogenes* (19)). 105 strains of *P. aeruginosa* and 43 strains of *H. influenzae* were also collected. For the Gram-positive cocci, staphylococci were in the greatest number: *S. aureus* (163) and CNS (112). We also studied 79 *S. pneumoniae* and 67 *E. fecalis*. Susceptibility tests were done according to the French CASFM recommendations. For the Enterobacteriaceae the main results are summarized in the following table.

	Amoxicillin	Amox-clav	Cefotaxim	Cefepim	Amikacin
% S	37	52	93	98	95
% I	1	16	2	2	2
% R	62	32	5	0	3

56% of the *P. aeruginosa* were susceptible to ticarcillin. 71% of the *S. aureus* were susceptible to methicillin.

P581 Nasopharyngeal carriage rate and susceptibility of *Haemophilus influenzae* to antimicrobials in central parts of Russia

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Objectives: To evaluate the rate of nasopharyngeal carriage of *Haemophilus influenzae* (Hif) among children 1–6 years old from the day-care centers in central regions of Russia and to elucidate the current resistance patterns of Hif.

Methods: A total of 733 children from Moscow, Smolensk and Yartstevo were included in this study. Nasopharyngeal cultures were collected using sterile cotton swabs. Isolates were identified with Gram stain, oxidase, and X+V factors. MICs were determined with Etest. Agents tested were ampicillin (AM), amoxycillin/clavulanate (XL), cefaclor (CF), erythromycin (ER), clarithromycin (CH),

roxithromycin (RO) and co-trimoxazole (TS). ß-Lactamase testing was performed with nitrocefin. Quality control was performed using *H. influenzae* ATCC 49247 and ATCC 49766.

Results: The total nasopharyngeal carriage rate of Hif was 44%, varying from 32% in Moscow to 46% and 55% in Yartstevo and Smolensk respectively. Only 2% of the strains were resistant to AM and 0.6% to XL and CF. Of 3 AM-resistant strains, 1 strain was ß-lactamase positive. Both ER and RO demonstrated very low activity against Hif (R%/1% were 12/88 and 97.9/2.1 respectively). The CH resistance rate was 18.7%. The highest level of resistance registered was for TS (20.9%).

Conclusions: There is a low prevalence of both ß-lactamase-positive and ß-lactamase-negative AM-resistant strains in children from central parts of Russia. AM remains the drug of choice for treatment of Hif infections. However, it is necessary to continue surveillance of AM resistance in participating regions. Of all tested macrolides, only CH had notable activity against Hif.

P582 Resistance to ceftazidime in a Hungarian hospital

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Objectives: The resistances of strains *of Escherichia coli, Klebsiella* and *Enterobacter* species to a panel of third-generation cephalosporins, aminoglycosides and ciprofloxacin were surveyed and the role of extended-spectrum beta-lactamases (ESBLs) studied.

Methods: Antibiotic sensitivity of the strains was determined with the disk diffusion method on Mueller–Hinton agar. ESBL were detected by the double-disk diffusion test and Etests. Genetic determinations of ESBL features were carried out by alkaline lysis of the bacterial cells, transformation and conjugation.

Results: Of about 600 *E. coli*, 140 *Klebsiella* and 450 *Enterobacter* strains, the ratios of resistant isolates ranged as follows: 7–13% of *E. coli*, 17–40% of *Klebsiella* spp. and 45–52% of *Enterobacter* spp.were resistant to third-generation cephalosporins.

5–11% of *E. coli*, 8–26% of *Klebsiella* spp. and 8–47% of *Enterobacter* spp. showed resistance to aminoglycosides. Further investigation of 106 ceftazidime-resistant or intermediate strains showed that they were resistant to cefoperazone, ceftriaxone, gentamicin, tobramycin and netilmicin, and sensitive to imipinem, meropenem, ciprofloxacin and amikacin. Nine out of 106 randomly selected ceftazidime-resistant strains proved to be ESBL producers. Successful transformations of plasmids from the ESBL-producing strains to *E. coli* DH5alpha resulted in resistance to ceftazidime, cefoperazone, ceftriaxone, tobramycin, gentamicin and netilmicin, while the transformed cells remained sensitive to carbepenems, ciprofloxacin and amikacin.

Conclusions: The survey has shown the simultaneous expression of the aminoglycoside resistance and the ESBLs.

P583 Antimicrobial resistance of enterobacteria isolated from minced meat

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Objectives: To find out if the high resistance levels in Gram-negative aerobic fecal flora are caused by high resistance levels in food. The use of antimicrobials in animal husbandry, and fecal contamination of carcasses at slaughter, could lead to the introduction of resistant strains of animal origin into the food-chain. Previous studies around the world have often found high resistance levels. Corresponding Finnish data have not been published.

Methods: At the local food surveillance laboratory, 26 minced meat samples (6 beef, 20 beef–pork) were plated onto MacConkey agar plates and incubated at 37°C. These were sent to the National Public Health Laboratory, where all colonies of different morphology were streaked for purity. Gram-negative, oxidase-negative, glucose-fermenting rods were further identified by a panel of 22 biochemical tests. In uncertain cases, four additional tests were done. In all, we attempted to identify 150 isolates to at least genus level. MICs were determined by the agar dilution method. NCCLS breakpoints were used. Duplicate isolates from the same sample were excluded on the basis of species identification and MICs, leaving 131 strains.

Results: The most common genera were *Serratia* (n=37), *Hafnia* (n=22), *Yersinia* (n=13), and *Citrobacter, Klebsiella* and unnamed enteric groups (n=10). Resistance to piperacillin/tazobactam, cefotaxime, aztreonam, imipenem, gentamicin, nalidixic acid and ciprofloxacin was 0%. Streptomycin resistance was 3.8%, chloramphenicol 0.8%, tetracycline 1.5%, sulfamethoxazole 1.5%, and trimethoprim 2.3%.

Conclusions: Resistance levels were low. Typically transferable resistance traits common in human strains were practically absent. Meat is not a major source of the high resistance levels in our fecal flora.

P584 The natural antimicrobial susceptibilities of *Enterobacter* spp.

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Objectives: The natural antimicrobial susceptibilities of five *Enterobacter* spp. were investigated, determining MIC values using a microdilution procedure.

Methods: The natural susceptibility of *Enterobacter* spp., i.e. '*E. cloacae*' group, *E. aerogenes*, *E. sakazakii*, *E. gergoviae* and *E. cancerogenus*, was tested against 71 antimicrobial agents. The strains examined were non-clonal and were obtained from different clinical and environmental sources in Germany. Identification results were confirmed using a commercial identification system. Antibiotic susceptibility tests were performed by microdilution in Isosensitest broth according to DIN 58940. The susceptibilities of the natural population were determined by analyzing the MIC distribution of all strains of one species for each antibiotic. This permitted determination of the biological thresholds, which limits the natural population with relatively low MICs from those strains with secondary (acquired) resistance. Whether the natural population was clinically sensitive, intermediate or resistant was determined by application of DIN standards.

Results: Among the five *Enterobacter* species, MIC distributions differed only slightly. They were naturally sensitive to all tested aminoglycosides, quinolones, modern penicillins, and third- and fourth-generation cephalosporins. *E. sakazakii* and *E. gergoviae* were the species most susceptible. Strains of the latter species were additionally sensitive to amoxycillin, amoxycillin/clavulanic acid, cefaclor, cefazolin and cefoxitin. All tested species were naturally resistant to penicillin G, oxacillin, glycopeptides, macrolides, clindamycin, lincomycin, nitrofurantoin, fusidinic acid and rifampicin.

Conclusions: The five clinically important *Enterobacter* species showed rather similar natural susceptibility patterns and were naturally sensitive to most of the antibiotics examined.

P585 Characterization of *Shigella* strains isolated from feces of children in Ifakara (Tanzania)

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Objectives: To epidemiologically type and to characterize the mechanisms of resistance of 84 strains of *Shigella* sp. isolated from children under 5 years of age in Ifakara, Tanzania during a diarrhea outbreak during the July–August period of 1996.

Methods: The epidemiologic relationships were established by REP-PCR and PFGE. MICs were determined using Etest. Mechanisms of resistance were studied using PCR, IEF, plasmid extraction, sequencing and detection of enzymatic activity of chloramphenicol acetyl transferase.

Results: Six epidemiologic groups were identified. Four of them contained the *S. flexneri* strains (90% of the strains) and the two remaining grouped separately *S. dysenteriae* and *S. sonnei*. 75% of the *S. flexneri* strains studied were resistant to ampicillin, due in 75% of the cases to the presence of an OXA-1 (pI=7), while the remaining 25% had a TEM (pI=5.4–5.6). 100% of the *S. dysenteriae* strains were ampicillin resistant and had the OXA-1, while only 25% of the *S. sonnei* were resistant and presented a TEM type beta-lactamase. No chromosomal beta-lactamases were detected. All strains studied were resistant to tetracycline, 65% were chloramphenicol resistant and 87% presented resistance to co-trimoxazole. *Dhfr*-type Ia and VII genes have been amplified. All isolates were susceptible to ciprofloxacin and nalidixic acid. Different integrons containing the OXA-1, the *dhfr* genes and an *aad* A2 have been amplified.

Conclusions: Three species of *Shigella* have been isolated from feces of children during a diarrhea outbreak. In general, the strains were highly resistant to tetracycline, ampicillin, chloramphenicol and co-trimoxazole, all of which are widely used in Africa.

P586 Susceptibility of 3134 consecutive bacterial isolates from infections to cefepime and nine other antibiotics. A multicenter study in seven eastern and central European countries

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Objectives: Increasing antimicrobial resistance is of great interest worldwide. However, there are few reports from eastern Europe.

Methods: The study was performed simultaneously in seven countries. From March 1997 to March 1998 consecutive clinical isolates were collected. Of the 3134 isolates, 976 were collected from six centers in Poland, 756 from five centers in the Czech Republic, 450 from three centers in Hungary, 300 from two centers in Slovakia, 301 from two centers in Lithuania, 221 from two centers in Estonia and 130 from Latvia. Susceptibility testing was performed with Etest (AB Biodisk, Sweden) and breakpoints were defined according to NCCLS standards. About 25% of the strains were Gram-positive cocci. 20% were isolates from blood.

Results: 57% of the isolates were from patients who were on any antibiotic in the last 2 weeks. These isolates were clearly more resistant than those from patients with no previous antibiotic treatment. There were no major differences in resistance between the countries. For one example (*E. coli*) the range of antibiotic resistance (in %) in the different regions was as follows: cefepime 0 –1.5, ceftazidime 1.3–6.7, cefotaxime 0–74, imipenem 0–1.5, PIP/TAZ 1.5–5.2, aztreonam 1–5.8, ciprofloxacin 0–7.8, ampicillin 33.8–49.6, amikacin 0–5.2, and gentamicin 2.6–10.8.

Conclusions: The results in this study give the impression of increased overall resistance compared to earlier findings in western Europe and definitely to resistance in Scandinavia. It is also nicely shown that earlier intake of antibiotics gives increased resistance.

P587 Antibiotic resistance of bacterial strains isolated from swimming pools in Greece

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Objectives: To study the distribution of antibiotic-resistant strains in swimming pool water.

Methods: Water samples from three swimming pools were examined for the total microbial flora at 22° C, total microbial flora at 37°C, total coliforms, fecal coliforms, *Enterococcus* spp., *Pseudomonas* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Mycobacterium* spp. and *Legionella* spp. One of the swimming pools was used only by children, one only by adults and one only by patients following water physiotherapy. The first two swimming pools were chlorinated, while the third was disinfected with ozone. All water samples were processed following the standard methods for the microbiological examination of water as suggested by APHA. Isolated bacterial strains were identified to the species level and sensitivity tests were performed following the Kirby–Bauer method.

Results: Multiresistant strains of *Pseudomonas alcaligenes*, *Micrococcus roseus* and *Staphylococcus aureus* were isolated from the children's swimming pool, *Staphylococcus aureus*, *Flavobacterium spiritovorum* and *Ochobactrum anthropi* were isolated from the adults' pool, and *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were isolated from the physiotherapy pool.

Conclusions: Swimming pool waters constitute an important reservoir of resistant bacterial strains. The currently used disinfection methods seem to be insufficient to protect public health effectively.

P588 Quantitative development of antibiotic resistance of *Pseudomonas aeruginosa* from patients with cystic fibrosis

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Objectives: Determination of changes in quantitative resistance of *Pseudomonas aeruginosa* from patients with cystic fibrosis.

Methods: 418 samples of 36 *Pseudomonas aeruginosa* strains with different PFGE patterns, collected from 33 chronically colonized children with cystic fibrosis in the period between April 1994 and April 1996, were investigated for their development of antibiotic resistance. During these 2 years the children received a combination of gentamicin and ceftazidime parenterally about every 3 months and in-between ciprofloxacin per os. The MICs of the 36 strains were measured against 12 antibiotics—gentamicin, amikacin, tobramycin, ciprofloxacin, levofloxacin, moxifloxacin, trovafloxacin, imipenem, meropenem, ceftazidime, cefepime and piperacillin—by means of broth microdilution tests according to DIN 58940.

Results: In the considered period of time, 15 strains developed a constant increase of the MICs. Six strains became resistant to gentamicin and amikacin, as well as to tobramycin in one case. With regard to the new fluoroquinolones, eight strains changed their susceptibility to moxifloxacin, four to trovafloxacin, and three to levofloxacin and ciprofloxacin. The MICs of imipenem changed in three strains, and in one strain that of meropenem as well. One strain became

resistant to piperacillin, two to ceftazidime and one to cefepime. A significant correlation between the MICs and the number of anti-pseudomonal courses of antibiotics was found for the aminoglycosides, meropenem, piperacillin, ciprofloxacin, and moxifloxacin.

Conclusions: Repeated courses of antibiotics in patients with cystic fibrosis are associated with increases in the MICs of the compounds used.

P588A Genomic variability and antibiotic resistance of sequential *Pseudomonas aeruginosa* isolates from bronchiectasis patients without cystic fibrosis

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Objectives: Most of the typing studies on *Pseudomonas aeruginosa* colonization and persistence in chronic lung diseases has been centered on cystic fibrosis (CF) patients. However, this bacterium also causes important infections in bronchiectasis patients without CF and it is beneficial to ascertain if it is the same or a different strain which reappears in the sputum after some time and/or antibiotic treatment

Methods: Thirty-seven sequential isolates from eight non-CF bronchiectasis patients were selected for between 1 and 3 years and characterized by two different PCR assays. Antibiotic susceptibility tests to aztreonam, amikacin, ceftazidime, ciprofloxacin, chloramphenicol, cefotaxime, cefuroxime, imipenem, meropenem and ofloxacin were done

Results: All patients had several hospital stays (range 7–49). It is of interest that four patients were found to harbor a mix of mucoid and non-mucoid isolates. Eleven different genotypic patterns were observed among isolates. Three of the eight patients were infected by two different strains, while five were each infected by the same strain. All the isolates, except one, were susceptible to carbapenems. Nine of 11 clones developed resistance to aztreonam and six to ciprofloxacin and ofloxacin. In all cases the last isolate of each patient showed the higher resistance level

Conclusions: *P. aeruginosa* infections in the patients studied were produced by a single clone or a maximum of two clones. Although there was phenotypic heterogeneity among the sequential isolates (mucoidity, different antibiotic resistance profiles), the genotypic pattern was the same. This suggests that the persistence of *P. aeruginosa* in chronic lung infections of bronchiectasis patients involves different phenotypic alterations that enhance its capacity to survive in this environment

Immunology, host defences, vaccination II

P589 The effects of streptomycin and ceftizoxime on humoral immunity in rabbits

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Antibiotics, in addition to killing or inhibiting the growth of microorganisms, may also affect the immune response in many ways. Therefore, immunomodulation of antibiotics is important, especially for immunocompromised transplantation and autoimmune patients.

We studied the effects of streptomycin (an aminoglycoside) and ceftizoxime (a cephalosporin) on rabbit humoral immunity. We injected 20 mg/kg/day streptomycin (group I), 17 mg/kg/day ceftizoxime (group II) and distilled water (control group) in two single doses for 14 days into adult male rabbits. In addition, SRBC used as antigen and a microhemagglutination laboratory method for evaluation of antibody production.

The results showed that specific lgM production was potentiated in primary and secondary responses in both groups: this was significant in group I on the 7th (37.6%) and 14th days (21.7%) ($p<0.01$), and in group II on the 7th, 14th and 21st days ($P<0.02$). On the other hand, specific IgG production in group I was enhanced significantly in primary responses ($P<0.02$), but no significant effects were seen in IgG secondary responses in the two groups.

Conclusion: These drugs potentiated primary immune responses, but there were no significant effects on secondary humoral responses, and it seems that these drugs act as adjuvants.

P590 Serum interferon-gamma and interleukin-10 in reactivation of cytomegalovirus infection during pregnancy

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Objectives: Reactivation of latent CMV infection may cause recurrent abortions. We aimed at finding out whether active CMV infection in pregnant women is associated with disturbed serum IFN-gamma and IL-10 levels.

Methods: The studies were performed on 12 women (mean age: 30 years) on the first day after consecutive spontaneous abortion in the second trimester of pregnancy, and in 15 women (mean age: 31 years) in the second trimester of a normal pregnancy (control group). Serum anti-CMV antibodies were determined by immunoenzymatic technique, employing Enzygnost AntiCMWIgM/IgG (Behring). Serum cytokine levels were established using human interferon-gamma ((h)IFN gamma), ELISA system (Biotrak) for IFN-gamma and human interleukin-10 ((h)IL- 10), and ELISA system for IL-10.

Results: In all women with obstetric failures an active CMV infection was diagnosed. In the experimental group, serum IFN-gamma levels were undetectable while serum IL-10 level averaged 5.0 ±2.0 μg/mL, which did not differ significantly from levels in the control group (p 0.05).

Conclusions: Reactivation of CMV infection in pregnancy may result from the absence of an IFN-gamma response.

P591 Granulocyte–macrophage colony-stimulating factor: prophylaxis of foreign body infection in a rat model

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Objectives: To determine the effect of granulocyte–macrophage colony-stimulating factor (GM-CSF) in a rat model of prophylaxis of foreign body infection.

Methods: One-cm polyurethane catheter segments were incubated for 2 h at 0°C in a suspension of a defined strain of *S. epidermidis.* Three catheters carrying a bacterial inoculum of 3.1 $\log_{10}$ CFU were implanted subcutaneously in each inbred Fisher rat. Murine GM-CSF was administered in a single dose of 4 mg/kg SC together with a single dose of teicoplanin 10 mg/kg IM 4 h before catheter implantation. In a second group, sterile water plus teicoplanin was given. The catheters were explanted after 72 h and quantitative catheter cultures were performed after sonication.